

HYDROGEN-ION CONCENTRATION OF ALBUMEN AND YOLK OF THE DEVELOPING AVIAN EGG

ALEXIS L. ROMANOFF

Agricultural Experiment Station, Cornell University, Ithaca, New York

The changes in hydrogen-ion concentration of the albumen and yolk in the avian egg have been considered as indicative of the character of the metabolic processes occurring within the egg. These changes are, therefore, of importance in studies of embryonic development (Needham, 1931; Romanoff and Hayward, 1943).

A review of the literature (Needham, 1931; Romanoff and Romanoff, 1929) indicates that considerable work has been done on the hydrogen-ion concentration of the hen's egg and very little on the eggs of other species (Shklyer, 1937). It is of interest to know whether or not the changes in this physical property of eggs are similar in different species of birds.

METHODS AND MATERIALS

To obtain these data the present study includes the eggs of the Leghorn chicken (*Gallus gallus*), Ring-necked pheasant (*Phasianus torquatus*), Bobwhite-quail (*Colinus virginianus*), White Holland turkey (*Meleagris gallopavo*), Pekin duck (*Anas platyrhynchos*) and of domestic goose (*Anser anser*). Particular effort was made to obtain eggs as fresh as possible. On an average the chicken eggs were one or two hours old, while the age of the eggs of other species varied from 24 to 36 hours. The pH value was determined electrometrically, using an hydrogen electrode. The observations were carried out: (1) on the albumen until it was merged with the yolk sac, thus losing its physical entity, and (2) on the yolk until hatching time.

EXPERIMENTAL RESULTS

Egg albumen

The data for hydrogen-ion changes in albumen (Fig. 1 *A*) show a striking similarity in all the curves. The initial rise in pH, from as low as 7.6 to as high as 9.5, at the beginning of incubation is followed first by a rapid, then by a more gradual decrease to approximate neutrality. All values obtained were for the middle dense layer of albumen, for it has been shown (Romanoff, 1943a) that the pH values of the different layers do not vary to any great extent even in fresh eggs. The results presented here agree with those of Shklyer (1937) for hens, turkeys, ducks and geese in all essentials except for the initial pH values which were higher than ours. Evidently the eggs used by Shklyer in his experiments were of more advanced age before their setting for incubation.

Egg yolk

Previous observations show that at certain stages of incubation there is a morphological differentiation of egg yolk into two fractions—dense and liquefied (Romanoff, 1943b), with quite distinct electrical conductivities (Romanoff and Grover,

1936). For that reason, as was anticipated, the dense egg yolk undergoes an entirely different change in hydrogen-ion concentration. There is a gradual rise from slight acidity, of about pH 6.0, to an alkalinity of about pH 7.8 near the end of the incubation period (Fig. 1 *B*). Then the pH values decrease slightly before the time of hatching. The data for all species follow the same general trend of change. This again is in close agreement with data published by Shklyer (1937).

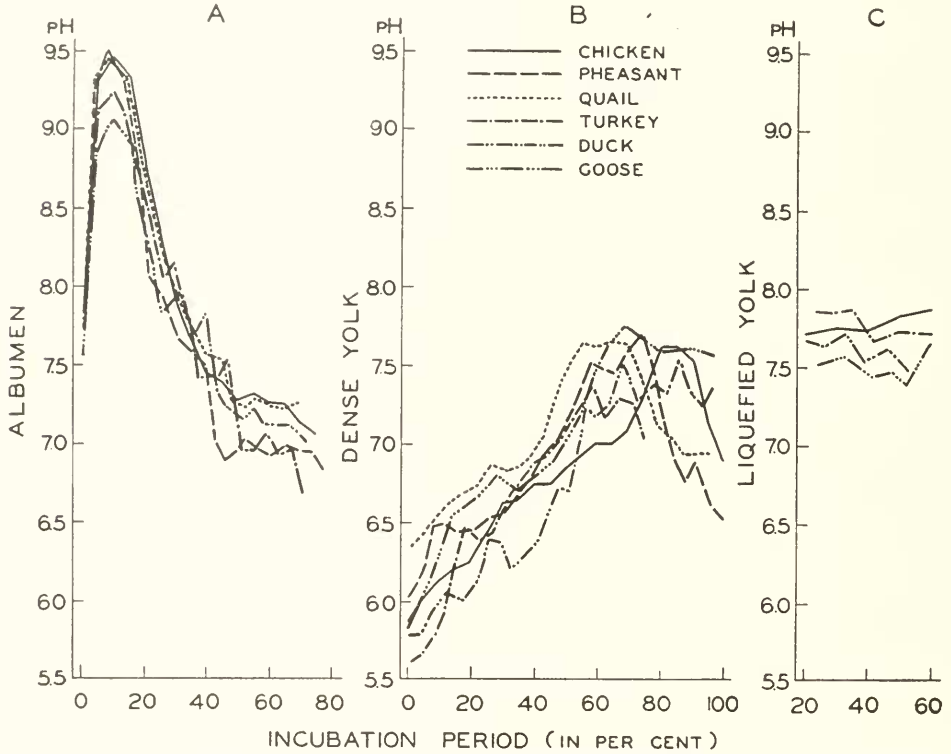


FIGURE 1. Changes in pH of avian eggs during embryonic development: *A*, albumen, *B*, dense yolk, and *C*, liquefied yolk. The data based on observations of over 600 eggs, daily averaging from 3 to 15 eggs for each species.

The liquefied yolk of avian eggs is consistently more alkaline in reaction than the semi-solid or dense portion (Fig. 1 *C*). According to Shklyer (1937) the liquefied yolk in the egg of the domestic fowl maintains an average pH value of about 7.7 throughout its period of existence.

DISCUSSION

It is now recognized that to obtain comparable results, certain variables must be controlled in ascertaining changes in the hydrogen-ion concentration of the developing egg; this is especially true of the albumen. Two of the most important of these variables are the age of the egg at the time of setting for incubation, and the conditions under which they have been kept in storage.

The pH value of the albumen increases rapidly in an egg with aging (Romanoff and Romanoff, 1929). Unless eggs are set for incubation immediately after laying, the initial rise in pH of albumen during the development may not be fully observed. For this reason the values for the incubated eggs given by other investigators (see reviews by Romanoff and Romanoff, 1929; Needham, 1931; and Shklyer, 1937) frequently show high initial pH value, which afterwards has only a steady decrease towards acidity. The older the egg at the beginning of the incubation the nearer the pH value will be to the peak of alkalinity in the initial stages. In eggs kept under ordinary environmental conditions (temperature about 12–13° C.) for only about seven days, the portion of the curve showing a rapid rise in pH would be almost completely eliminated. Consequently without adequate control of the age of the egg, the results of many former studies demonstrate either only a very slight rise in the curve (Gueylard and Portier, 1925; Penionschkevitch, 1934; Berenstein and Penionschkevich, 1935) or none at all (Aggazzotti, 1913; Buytendijk and Woerdeman, 1927; Shklyer, 1937).

The initial rapid rise in the pH of the albumen during early incubation, as well as in storage, has been shown to be caused by the loss of carbon dioxide (Sharp and Powell, 1931; Brooks and Pace, 1938). It has been determined experimentally that the pH of the egg albumen is in direct relationship to the concentration of carbon dioxide in the incubator (Romanoff and Romanoff, 1930, 1933). With 10 per cent of carbon dioxide in the air the pH value of albumen does not rise at all—the curve flattens out, and the normal peak of high alkalinity is not observed. Similarly in storage at low temperature (0° C.), the rise in pH may be prevented by high carbon dioxide pressure (Moran, 1937).

It is the author's experience that with the eggs of the same preincubation age, the incubating temperature, within the range of embryonic survival, 35.5–39.5° C. (Romanoff, Smith and Sullivan, 1938), has a very insignificant effect on the variation in hydrogen-ion concentration of albumen and yolk. Also, negative results were obtained with the changes of relative humidity in the incubator (Penionschkevitch, 1934). However, according to Sharp and Powell (1931), the rise in pH of egg albumen prior to incubation is hastened by a higher temperature.

SUMMARY

The observations on incubated eggs of chicken, pheasant, quail, turkey, duck, and goose clearly indicate that changes in hydrogen-ion concentration of albumen, and of dense and liquefied portions of yolk are similar for all species studied, and suggest a pattern which may be characteristic of all avian eggs. In the albumen there is a rapid rise in pH, then a fall; in the dense yolk, a gradual rise with a slight fall at hatching; and in the liquefied yolk during its existence, high pH value without change.

The initial pH value of egg albumen during embryonic development depends chiefly upon the preincubation age of the egg—fresh eggs would give low, while older eggs would give high pH values of albumen at the beginning of incubation.

LITERATURE CITED

- AGGAZZOTTI, A., 1913. Influenza dell'aria rarefatta sull'ontogenesi; Nota II. La reazione dei liquidi dell'ovo durante lo sviluppo. *Arch. f. Entomoch. Org.*, 37: 1–28.

- BERENSTEIN, F. J., AND E. E. PENIONSCHKEVITCH, 1935. Ueber die aktive Reaktion des Inhalts der Hühner- und Enteneier während der Inkubationsperiode. *Physiol. Jour.* (U.S.S.R.), **18**: 654-659.
- BROOKS, J., AND PACE, 1938. Distribution of carbon dioxide in the hen egg. *Proc. Roy. Soc. London, B*, **126**: 196-210.
- BUYTENDIJK, F. J. J., AND M. W. WOERDEMAN, 1927. Die physico-chemischen Erscheinungen während des Eientwicklung. I. Die Messung der Wasserstoffionenkonzentration. *Arch. f. Entwmech. Org.*, **112**: 387-410.
- GUEYLARD, F., AND P. PORTIER, 1925. Réaction ionique des différents constituants de l'oeuf de la poule. Ses modifications au cours de l'incubation. *Compt. Rend. Acad. Sci.*, **180**: 1962-1963.
- MORAN, T., 1937. Gas storage of eggs. *Jour. Soc. Chem. Ind.*, **56** (T.): 96-101.
- NEEDHAM, J. 1931. *Chemical embryology*. Cambridge University Press.
- PENIONSCHKEVITCH, E. E., 1934. Die Veränderung der aktiven Reaktion (pH) des Eiweisses und Dotters der Hühnereier bei verschiedenen Brutbedingungen. *Archiv. f. Geflügelkunde*, **8**: 273-280.
- ROMANOFF, A. L., 1943a. Assimilation of avian yolk and albumen under normal and extreme incubating temperatures. *Anat. Rec.*, **86**: 143-148.
- ROMANOFF, A. L., 1943b. Morphological and physiochemical differentiation in various layers of avian albumen. *Food Research*, **8**: 286-291.
- ROMANOFF, A. L., AND H. J. GROVER, 1936. Electrical conductivity of yolk, albumen, allantoic and amniotic fluids of the developing birds' eggs. *Jour. Cell. and Comp. Physiol.*, **7**: 425-431.
- ROMANOFF, A. L., AND F. W. HAYWARD, 1943. Changes in volume and physical properties of allantoic and amniotic fluids under normal and extreme temperatures. *Biol. Bull.*, **84**: 141-147.
- ROMANOFF, A. L., AND A. J. ROMANOFF, 1929. Changes in pH of albumen and yolk in the course of embryonic development under natural and artificial incubation. *Biol. Bull.* **57**: 300-306.
- ROMANOFF, A. L., AND A. J. ROMANOFF, 1930. Effect of carbon dioxide on pH of albumen in the developing hen's egg. *Jour. Exp. Zool.*, **56**: 451-457.
- ROMANOFF, A. L., AND A. J. ROMANOFF, 1933. Biochemistry and biophysics of the developing hen's egg. II. Influence of composition of air. Cornell Univ. Agr. Exp. Sta. Memoir 150: 1-36.
- ROMANOFF, A. L., L. L. SMITH, AND R. A. SULLIVAN, 1938. Biochemistry and biophysics of the developing egg. III. Influence of temperature. Cornell Univ. Agr. Exp. Sta. Memoir 216: 1-42.
- SHARP, P. F., AND C. K. POWELL, 1931. Increase in the pH of the white and yolk of hens' eggs. *Ind. Eng. Chem.*, **23**: 196-199.
- SHKLYER, N. M., 1937. A study of physico-chemical changes in the egg during embryonic development of birds. I. Changes in the concentration of hydrogen-ion in relation to embryonic development in the eggs of domestic fowl (hens, turkeys, ducks, geese). *Ukrainian Biochem. Jour.*, **2**: 379-406.